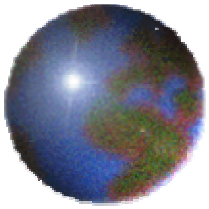
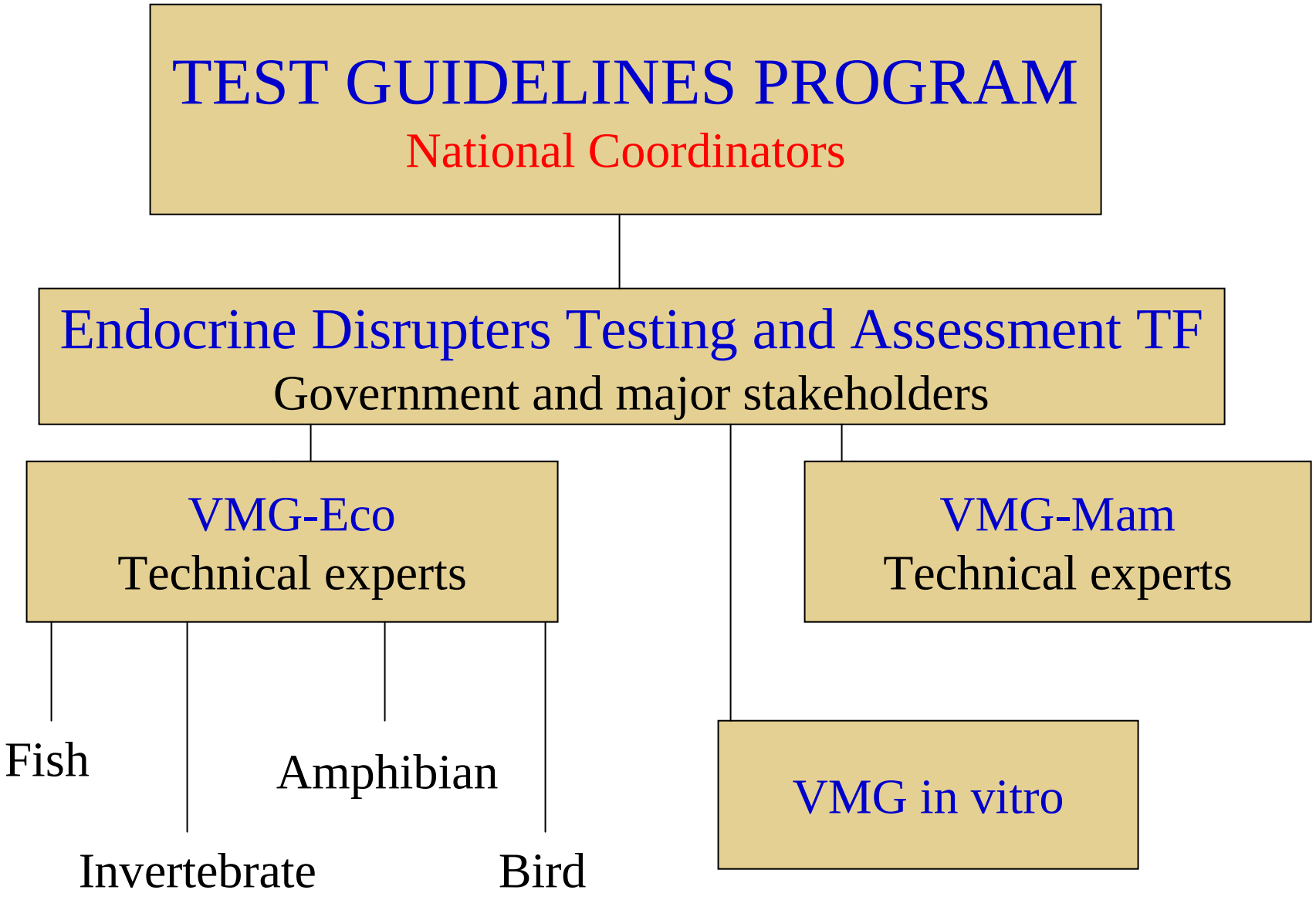
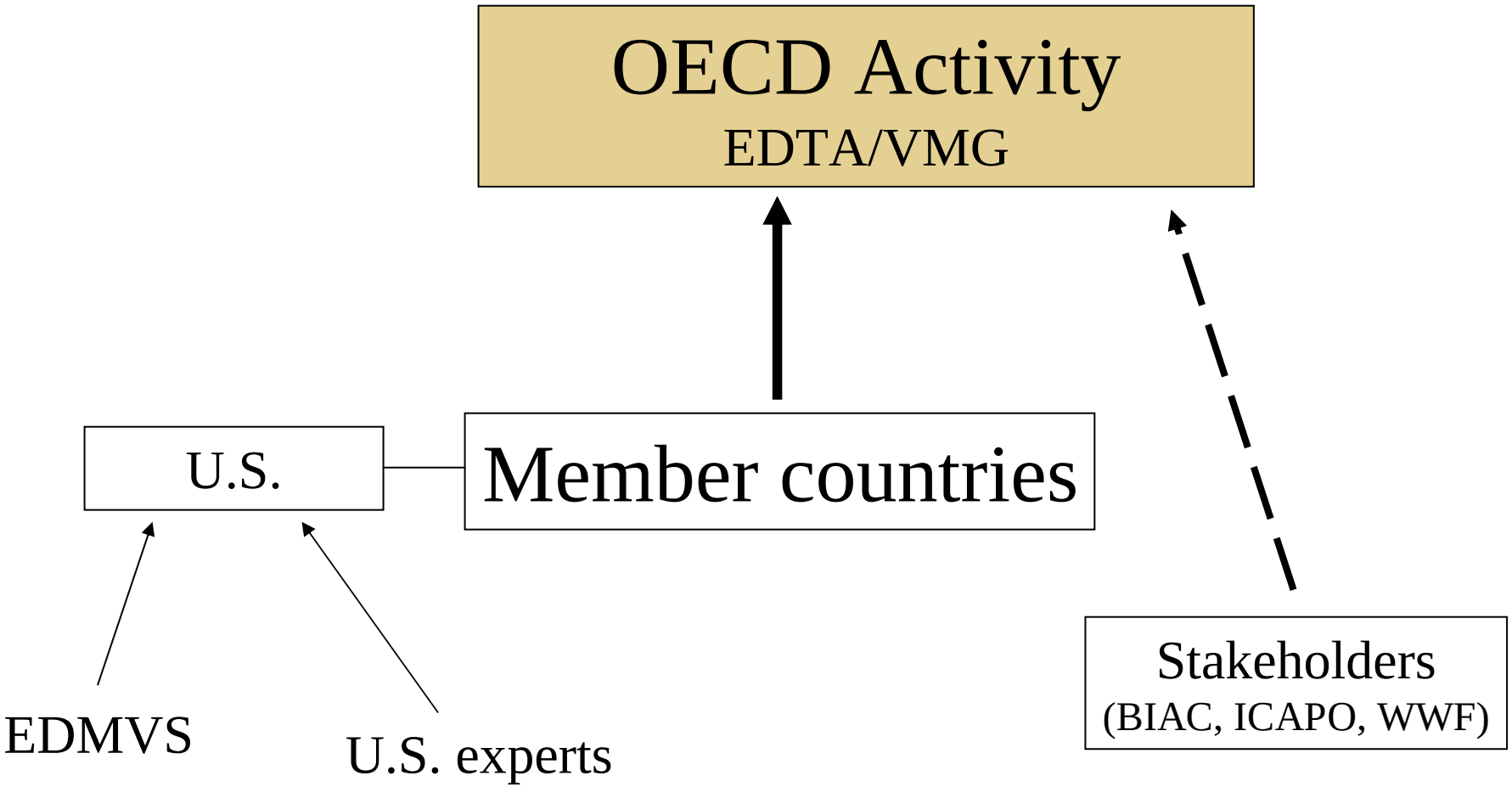


2003 OECD Activities

Gary Timm and Les Touart

OECD ED Test Guideline Roles




OECD Activity
EDTA/VMG
U.S.
Member countries
Stakeholders
(BIAC, ICAPO, WWF)
EDMVS
U.S. experts

Possible Modes of OECD Involvement in Test Method Validation

1. Coordinates prevalidation and validation
2. Lead country develops prevalidation data; OECD coordinates and directs validation.
3. Lead country develops all prevalidation and validation data for OECD guideline development
4. OECD facilitates information exchange; no OECD test guideline development.

Process for International Guidelines

- Follow ICCVAM requirements for method validation
- OECD's Endocrine Disruptor Testing and Assessment workgroup will be primary vehicle for deliberation and stakeholder input for modes 1 and 2 on previous slide
- EDMVS will be kept informed and will be asked for input for US position

Process for International Guidelines

- US will be lead country on most guidelines
  - Lead country coordinates technical work
  - US volunteering for lead country because we have resources and are mandated to meet schedule
- Peer review by letter
- National Coordinator comment process

Role of EDTA

- Plan and execute pre-validation and validation of endocrine test procedures
- Oversee the development of test guidelines based on the validated procedures
- Provide review and quality control of documents prior to submission to National Coordinators

2003 OECD Meeting Schedule

- VMG non animal (March 17-18)
- VMG mammalian (April 14-15)
- VMG eco (May 2)
  - Amphibian Expert Group (June 26-27)
  - Fish Drafting Group (May 3, September)
  - Invertebrate Expert Group (October)
  - Avian Expert Group (Spring ’04)
- EDTA (May 22-23)

VMG non animal (VMG-NA) Agenda

- Receptor Binding Assays
- Aromatase and Steroidogenesis Assays
- Reporter Gene/Transcriptional Activation Assays
- Discussion of QSARs
- In Vitro Cell/Tissue Assays

VMG-NA Conclusions and Commitments

- **Receptor binding assays:**
  - US will coordinate the development and validation of recombinant ER and AR assays
  - Switzerland and CERI (Japan) are interested in participating in the validation of this assay.
- **Steroidogenesis and aromatase:**
  - US will pursue development of a cell-based assay using H295R cell line.
  - Japan will continue the development of an aromatase assay with the KGN cell line.

VMG-NA Conclusions and Commitments (2)

- Reporter gene assays:
  - Japan has completed prevalidation of ERTA system. Protocol for validation and chemical list will be available at next VMG meeting.
  - Patent issues. What would validation consist of?
  - Japan also developing ERβ and AR assays.
- Thyroid:
  - US will revise and complete ICAPO DRP on in vitro thyroid methods
  - CERI will run a version of the FRLP-5 cell line with T3

VMG mammalian Agenda

- Validation of the uterotrophic assay
- Validation of the Hershberger
- Validation of the enhanced OECD 407
- Thyroid hormone testing
- Sharing the work on testing and assessment

Logic of OECD Mammalian Validation Approach

- VMG adopts initial protocol
- Lead lab and participating labs identified
- Phase 1 design
  - All labs perform protocol with strong positive chemical
  - Check lab competence and endpoint responsiveness
  - Refine protocol as necessary for phase 2 trials
- Phase 2 design
  - Challenge protocol with weak acting chemicals and negatives
  - Conduct blind trials
  - Measure interlaboratory variability

Uterotrophic Assay

- An in vivo assay to detect estrogens
- Phase 1
  - 4 protocols tested: immature oral, immature sc, ovariectomized 3 day sc, OVX 7 day sc.
  - 19 laboratories; ethynyl estradiol (EE).
- Phase 2
  - EE was used as reference positive at two doses
  - Dose response study using 5 weak estrogens: bisphenol A, o,p'-DDT, genistein, methoxychlor, nonylphenol

Uterotrophic Assay (2)

- Coded single-dose study
  - Used same five weak agonists
  - Dibutyl phathalate included as negative chemical
  - EE included as strong positive
- Conclusions
  - The uterotrophic assay is reliable and relevant
    - All protocols detected each of the five weak estrogens
    - Four different comparisons of intra and interlaboratory variability were made

Uterotrophic Assay (3)

- No protocol was consistently superior
  - SC usually more sensitive than oral except when first pass metabolism was required for activation
  - No strain differences or differences in diet noted so long as phytoestrogen intake did not exceed 50 mg/kg/day (sensitivity to phytoestrogens: OVX rats< immature rats< mice)
- Recommend peer review
  - Independent peer review panel; solicited by NC
  - Review by mail and conference call
  - Transparent process

Hershberger Assay

- An in vivo assay to detect androgens and antiandrogens
- Phase 1
  - 17 laboratories; testosterone propionate (androgen), flutamide (antiandrogen)
- Phase 2
  - Testosterone propionate is the reference androgen
  - AR agonists: TP, 17α-methyltestosterone, trenbolone
  - AR antagonists: vinclozolin, procymidone, linuron, p,p'-DDE, flutamide

Hershberger Assay (2)

- 5α-Reductase Inhibitor: Finasteride
- Status
  - Phase 1 completed 2001
  - Phase 2 completed May 2003
  - Phase 3 planned to complete validation by Dec 2003
  - Peer review planned for March 2004
- Phase 3
  - Testing coded chemicals
  - Testing a negative chemical
  - Compare weanling males with castrated males

Enhanced OECD 407

- OECD 407
  - 28 day repeat-dose study in adult rodents
  - 3 dose levels plus control
  - Used in EU and Japan for new chemicals and as general tox screen in tiered testing
- ED endpoints
  - Organ wts. (sex organs, accessory tissues, thyroid)
  - Histopathology
  - Spermatology (motility, morphology, number)
  - Hormone measurements

Enhanced OECD 407 (2)

- Phase 1
  - 7 laboratories performed phase 1 studies. Flutamide or other strong endocrine disruptor
  - Conclusions from phase 1 were to drop sperm motility and non-thyroid hormone measures
- Phase 2
  - 10 chemicals each to be tested in 2 laboratories:
    - 6 strong: Ethynyl estradiol, tamoxifen, methyl testosterone, flutamide, L-thyroxine, and PTU
    - 4 weak: CGS 18320B1; p,p'-DDE; genestein, nonyl phenol
  - Split design: two groups of 5 animals

Enhanced OECD 407 (3)

- Status and next steps
  - Laboratory studies complete Dec 2002
  - Data compilation complete May 2003
  - Draft final report December 2003

VMG ecotoxicity Agenda

- Validation of the Fish Screen
- Fish Testing (Tier II)
- Validation of an Amphibian Screen
  - Thyroid testing
- Avian Testing
- Invertebrates

Fish Screen

- Vitellogenin method comparisons
  - Fathead minnow method survey completed
  - Zebrafish and medaka method surveys in progress
- OECD Phase 1A
  - All three species (fathead minnow, zebrafish, medaka)
  - Non-spawning method (21-day)
  - Three endpoints (Vtg, GSI, gonad histopathology)
  - Test chemicals - 17β-estradiol and trenbolone
- Comparative Evaluation of Assay Methods
  - Fathead minnow only
  - Reproduction assays (14 day & 21 day) and Non-spawning (14 day)
  - Test chemicals – methoxychlor, trenbolone,flutamide,fadrozole
- Partial life cycle assay
  - Proposed by Denmark, given high priority by NCs
  - 60-90 day test, key endpoint = sexual differentiation

Fish Screen – next steps

- Fish Drafting Group meeting in September
  - Decide on assay method
  - Plan Phase 1B – multichemical evaluation of selected assay
  - Select chemicals for Phase 1B

Fish Testing (Tier II)

- Fish Two-generation Test DRP (US) and Fish Two-generation TG (US) circulated for comment
- Fish Life Cycle TG (Japan) circulated for comment
- Fish Drafting Group (meeting in September)
  - Plan Phase 1A of validation for fish testing

Amphibian Screen

- Revision of the amphibian screen DRP
- International workshop to discuss amphibian methods (24-25 June)
- Amphibian Expert Group meeting (26-27 June)
  - Recommend single assay to advance through validation

Thyroid Hormone Disruption Assays DRP

Purpose to consider the applicability of existing assays under development, suggest alternative approaches, and identify additional data needs to facilitate a consensus direction.

Avian Testing

- Avian two-generation toxicity test DRP circulated for comment
- Avian dosing study in progress
- Additional activities
  - One generation (proven breeder) method
  - Two generation species comparison study (Japanese quail & bobwhite quail)
  - Avian embryo assay (range finding method)

Invertebrates

- Recommend revising Mysid Life Cycle DRP to include Crustaceans in general
- Recommend invertebrate testing assays be expanded to include annelids, molluscs, and echinoderms in addition to arthropods
- Recommend considering appropriate invertebrate screening assays – focus on the "most sensitive" taxa